

Mycorrhizal Status of *Narcissus papyraceus* Ker-Gawl. Co-Cultivated With *Cynodon dactylon* Pers.

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ABSTRACT

Arbuscular mycorrhizal (AM) status of paper white (*Narcissus papyraceus* Ker-Gawl.) cultivated in a grassy lawn was studied at three growth stages viz. early and late vegetative, and flowering stage. Effect of *N. papyraceus* on mycorrhizal status of co-cultivated grass (*Cynodon dactylon* Pers.) was also studied. A difference in mycorrhizal colonization of *N. papyraceus* was observed at the different growth stages. Extent of mycorrhizal colonization was 92 cm 100⁻¹ cm of root length at flowering stage as compared to 53 and 48 cm/100 cm at early and late vegetative growth stages, respectively. Highest percentage colonization of mycelium and arbuscules i.e., 89 and 86%, respectively was recorded at flowering stage and was significantly different than colonization at the two vegetative growth stages. Number of arbuscules varied from 83/10 cm of root length at flowering stage to 12 and 13 at early and late vegetative growth stages, respectively. In contrast to that vesicular colonization did not show any difference among the growth stages. *C. dactylon* roots were fairly colonized with mycorrhiza showing 64, 6 and 33% mycelial, arbuscular and vesicular colonization, respectively in non-rhizospheric soil of *N. papyraceus*. *C. dactylon* plants growing in the rhizospheric soil of *Narcissus* exhibited higher arbuscular and vesicular colonization as compared to non-rhizospheric soil.

Key Words: Arbuscular mycorrhizae; *Cynodon dactylon*; *Narcissus papyraceus*

INTRODUCTION

Arbuscular mycorrhizae play important role in terrestrial ecosystems, such as grasslands, where they influence plant community structure and nutrient cycling (Jackson & Mason, 1984). Mycorrhizal association enhances plant growth and productivity by increasing nutrient element uptake (Al-Karaki, 2002). They also impart other benefits to plants including enhanced enzymatic production (Adriano-Anaya *et al.*, 2006), increased rate of photosynthesis (Wu & Xia, 2006), enhancement of nitrogen fixation by symbiotic or associative N₂-fixing bacteria (Javaid *et al.*, 1994; Antunes *et al.*, 2006), osmotic adjustment under drought stress (Ruiz-Lozano, 2003), increased resistance to pests (Whipps, 2004), tolerance to various abiotic stress factors (Javaid & Bajwa, 1999; Takeda *et al.*, 2007) and improving soil aggregation (Rillig & Mumme, 2006) and thus improved soil physical properties and stability. Besmer and Koide (1999) attributed the increased vase-life of cut flowers of *Antirrhinum majus* L. to the reduction of ethylene production in mycorrhizal plants.

Cultivation of non-conventional crops have great prospective to meet financial needs of farming community. In this regard ornamental plants especially cut flowers have attracted farmers globally. Cut flowers represent the important segment of the floriculture industry. Floriculture is an emerging sector in Pakistan. *Narcissus* is one of the cut flowers, which are being cultivated globally due to their medicinal and ornamental values. The species of *Narcissus*

are mostly native to the Mediterranean region, but a few species are found from Central Asia to China. *Narcissus papyraceus*, commonly known as Paper white narcissus, is a perennial bulbous plant. The white flowers are produced on bunches and are fragrant. It is frequently grown as a house plant and cut flower on commercial scale in Pakistan (Anonymous, 2003). The present study was designed to evaluate the mycorrhizal status of *N. papyraceus* grown in a grassy lawn for ornamental purpose. The study was also extended to investigate the effect of *N. papyraceus* on the mycorrhizal status of co-cultivated *Cynodon dactylon*.

MATERIALS AND METHODS

Sampling of plant materials. The bulbs of *N. papyraceus* were planted in a grassy lawn in Mycology and Plant Pathology Department, Punjab University Lahore (Fig. 1). Its roots were collected at three growth stages viz. early vegetative, late vegetative and flowering stage. Similarly root of *C. dactylon* were collected from the rhizospheric and non-rhizospheric soils of *N. papyraceus*. Roots were thoroughly washed under tap water and fine roots of both the test plant samples were cut into 1 cm pieces.

Clearing and staining of roots. After careful rinsing with tap water, the root samples were cleared and stained for AM study following Phillips and Hayman (1970). The roots were cleared for about 30 min in 10% KOH solution in an autoclave, placed in 10% HCl for 10 min for neutralization and then stained with 0.05% glycerol-trypan blue solution.

Arbuscular mycorrhizal study. Randomly selected, 20

stained root pieces of 1 cm each were studied for each plant sample. Root pieces were mounted in lactophenol on glass slides and studied under compound microscope. For percentage mycelial, arbuscular and vesicular colonization, each root piece was observed at 5 points under $\times 10$ of a compound microscope. The percentage occurrence of various AM structures viz. mycelium, arbuscules and vesicles was recorded on the bases of their presence or absence. Arbuscular and vesicular colonization were quantified by counting these structures per 10 cm of root length. The extent of mycorrhizal colonization was quantified in terms of $\text{cm } 100^{-1} \text{ cm}$ of root length.

Statistical analysis. Data regarding the effect of various growth stages on mycorrhizal colonization was subjected to one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Steel & Torrie, 1980) to separate the means. Data regarding the effect of *N. papyraceus* on the mycorrhizal colonization of co-cultivated *C. dactylon* was analyzed by applying t-test.

RESULTS AND DISCUSSION

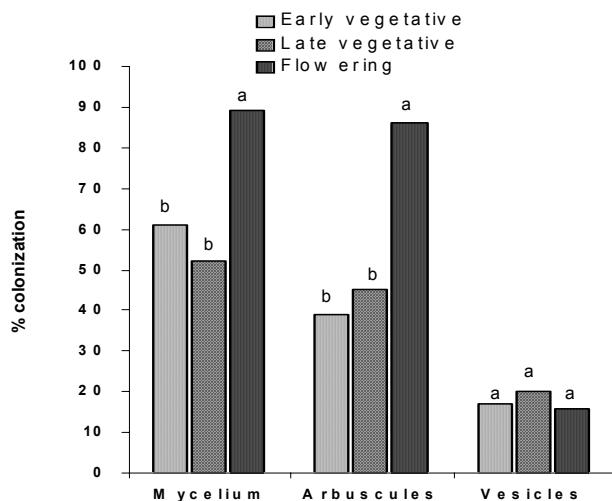
Mycorrhizal status of *N. papyraceus*. Highest and significantly greater mycelial and arbuscular colonization was recorded at flowering stage as compared to either early or late vegetative growth stage. There was 89% mycelial colonization at flowering stage as compared to 61 and 56% at early and late vegetative growth stages, respectively (Fig. 2). Extent of mycorrhizal colonization was 53, 48 and 92 $\text{cm } 100^{-1} \text{ cm}$ of root length at early and late vegetative and flowering stages, respectively. Number of arbuscules per 10 cm of root length was 83 at flowering stage that was significantly greater than the number of arbuscules at both early and late growth stages (Fig. 3). The most important function of arbuscular mycorrhizal fungi is thought to be the nutrient absorption from the soil to enhance the crop growth and yield (Smith & Read, 1997). The increase in mycorrhizal structures viz. mycelium and arbuscules at flowering stage clearly indicates that mycorrhizal colonization in *N. papyraceus* plays an important role in meeting the enhanced nutrient requirements at this stage. The intraradical mycelium of the root cortex also extends from the root out into the soil, where they interface with soil particles. These extraradical hyphae function as absorptive structures for mineral elements and water. Since they can extend out several centimeters from the roots, they can effectively bridge over the zone of nutrient depletion around roots and absorb immobile elements from the bulk soil (Bethlenfalvay & Linderman, 1992). Arbuscules are the structures, where metabolites exchanges take place between the fungus and host cytoplasm (Parniske, 2000). The results of the present study reveal the possibility of enhanced nutrient accretion by enhanced mycelial colonization and the enhanced metabolic exchanges between the symbiotic partners due to increased arbuscular colonization at flowering stage. In the present study there was not any

Fig. 1. *Narcissus* plants growing in a grassy lawn



Fig. 2. Percentage mycorrhizal colonization in roots of *Narcissus* at different growth stages.

Values with different letters show significant difference as determined by Duncan's Multiple Range Test.

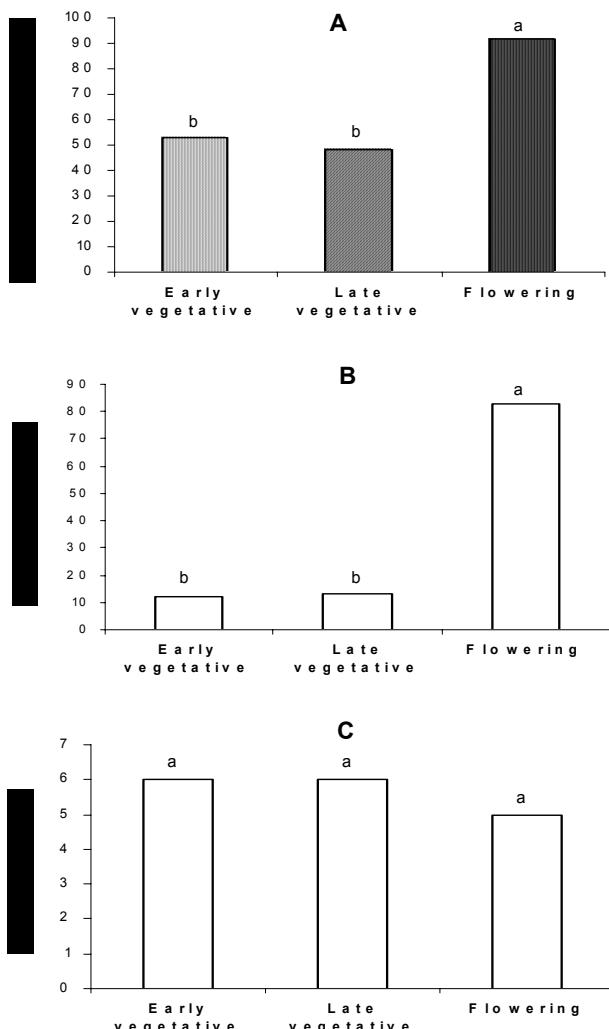


pronounced difference in vesicular colonization among the growth stages (Fig. 2 & 3). It could be attributed to the fact that vesicles usually form later as terminal or intercalary swellings in the cortical cells and function as nutrient storage organs or as propagules in root fragments (Smith & Read, 1997) and thus their number is important towards the end of the crop growing season and less important up to flowering stage.

Mycorrhizal status of *C. dactylon*. Root of *C. dactylon*, were fairly colonized by mycorrhizal fungi. There was 64, 6 and 33% mycelial, arbuscular and vesicular colonization, respectively and 57 $\text{cm } 100^{-1} \text{ cm}$ colonization extent in root cortex of *C. dactylon* plants growing away from the rhizosphere of *N. papyraceus* (Fig. 4). AM status of grasses has been studied with great contradictory results. Since grasses have fibrous and highly branched root systems and are relatively efficient in nutrient absorption, hence are generally considered to be only weakly dependent on

Fig. 3. Extent of mycorrhizal colonization, and number of arbuscules and vesicles in roots of *Narcissus* at different growth stages

Values with different letters show significant difference as determined by Duncan's Multiple Range Test



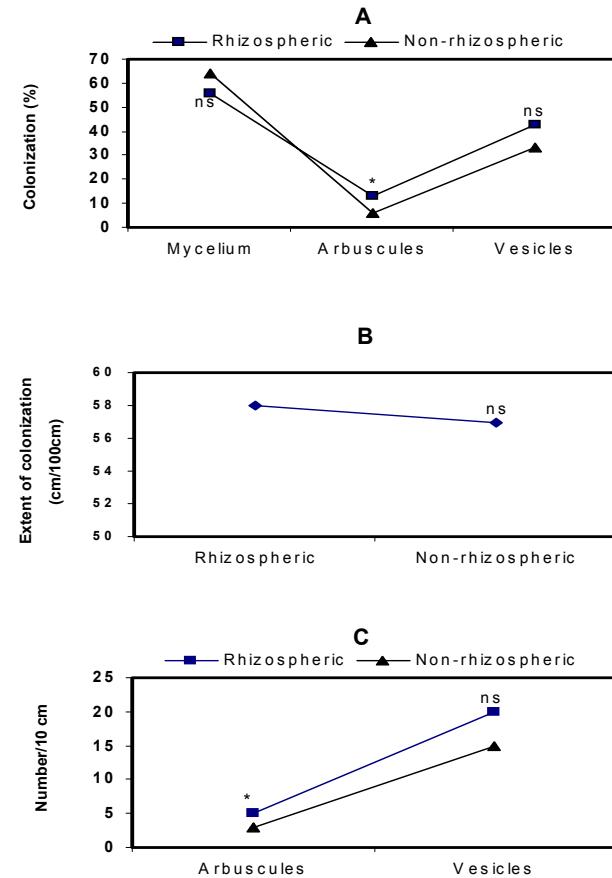
mycorrhizal symbiosis for nutrient acquisition (Baylis, 1974; Janos, 1980). In contrast, grasses were regarded as heavily mycorrhizal ones by Nicolson (1960), Nicolson and Johnston (1979) and De La Pena *et al.* (2006). Recently, Anjum *et al.* (2006) studied the mycorrhizal status of different rainy season grasses collected from Lahore and found that there was not any correlation between plant growth and mycorrhizal colonization of these grasses.

Arbuscular and vesicular colonizations were enhanced in the rhizosphere of *N. papyraceus* (Fig. 4). Earlier Javaid *et al.* (1995) reported that root colonization with AM fungi was enhanced in leguminous plant (*Trifolium alexandrinum* L.) when grown in mixed culture with *B. campestris*. Similarly Ocampo *et al.* (1980) observed enhanced AM colonization in onion when grown with Swedes than when grown alone and similar results have also been obtained in

Fig. 4. Mycorrhizal colonization in roots of *Cynodon* in rhizospheric and non-rhizospheric soils of *Narcissus*

ns: Non-significant difference between rhizospheric and non-rhizospheric soils.

* Significant difference between rhizospheric and non-rhizospheric soils as determined by t-test.



barley, when grown in mixed culture with rape. Iqbal and Rana (1991) found that weed species were heavily mycorrhizal when grown with *B. campestris* in biologically reclaimed saline soil.

CONCLUSION

Mycorrhizal fungi may have some important role at flowering stage in *N. papyraceus*. The findings of the study highlight the need for detailed investigation of the relationship between vase-life and mycorrhizal colonization as mycorrhizal fungi play an important role in increasing vase-life of cut flowers by reducing ethylene production (Besmer & Koide, 1999).

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